

Lectin Binding in Membranoproliferative Glomerulonephritis

Evidence for N-Acetylglucosamine in Dense Intramembranous Deposits

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Type II membranoproliferative glomerulonephritis (MPGN-II) is characterized by electron-dense intramembranous deposits (DIMD) in the basal laminae of the kidney. These deposits selectively bind the lectin wheat germ agglutinin (WGA) or its succinylated derivative. In renal tissue samples from normal controls, Type I membranoproliferative glomerulonephritis, and several other renal diseases, only a normal pattern of WGA binding

was observed and no membrane-oriented deposits reacted with WGA. Additional attempts to stain the DIMD with any of eight other lectins or eight antisera to renal antigens were uniformly unsuccessful. Discrete WGA reactivity indicates that the deposits contain appreciable quantities of internally linked N-acetylglucosamine and may also provide a valuable adjunct in making the histologic diagnosis of MPGN-II. (*Am J Pathol* 1985, 118:325-330)

IN 1961, Berger and Galle described the unique appearance of electron-dense deposits occurring in the basal laminae of three renal biopsies.¹ Since that initial observation, nephrologists and pathologists have recognized the existence of a second type of membranoproliferative glomerulonephritis (MPGN-II) which is virtually indistinguishable from Type I MPGN on the basis of clinical criteria alone. The hallmark of MPGN-II is the typical dense intramembranous deposit (DIMD) observed by electron microscopy.²

Considerable confusion in terminology arose because, unlike the usual dense deposits of immune complex renal disease, these unique DIMDs did not appear to contain immunoglobulin.^{2,3} The deposits have to date defied both immunologic and precise biochemical definition. Also unlike immune complexes, only the surface of the DIMD appears to bind the third component of complement (C3) and the terminal complement components.⁴ This leads to a characteristic finding by immunofluorescence microscopy, with C3 outlining the DIMD and producing the appearance of basement membrane "railroad tracks" and mesangial "rings."³

By descriptive histology these DIMDs appear refractile when large enough, stain brightly with periodic acid-Schiff (PAS) and are quite osmophilic, which leads to their electron-dense appearance. Biochemical studies have shown that quantitatively more sialic acid and less cystine is present in isolated basement membranes from these kidneys,⁵ but no other biochemical abnormalities have been defined.

Lectins encompass a remarkable family of proteins and glycoproteins which selectively bind to certain carbohydrates.^{6,7} Because lectins exhibit very discrete carbohydrate binding and the carbohydrates are relatively resistant to histologic fixation, labeled lectins are powerful reagents to define both normal and altered tissue distribution of carbohydrates. Recently, lectins have been used for isolating^{8,9} identifying,¹⁰⁻¹³ and purifying glycoproteins.¹⁴ Labeled lectins have also been used for examining the histologic distribution of carbohydrates in both animal^{15,16} and human renal tissues.^{17,18}

The present study reports the specific binding of wheat germ agglutinin (WGA) or its succinylated derivative to the dense deposits of MPGN-II. Since WGA is known to bind N-acetylglucosamine (NAG) and its polymers, this observation suggests that large quantities of NAG are present in DIMDs.

Materials and Methods

Reagents

Lectins labeled with either fluorescein isothiocyanate or tetramethyl rhodamine isothiocyanate were obtained

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from Vector Laboratories, Inc. (Burlingame, Calif) and included concanavalin A (ConA) (lot #81107), WGA (lot #91020), *Ricinus communis* I (lot #70719), peanut agglutinin (PNA) (lot #81113), *Ulex europaeus* agglutinin I (UEA) (lot #81007), *Dolichos biflorus* agglutinin (DBA) (lot #70716), soybean agglutinin (lot #81115), and succinylated WGA (lot #91022).

Bandeiraea simplicifolia II (catalog #L-1259, lot #119C-3927), *B simplicifolia* I-B₄ (catalog #L-1134, lot #119C-3925) and NAG (lot #32F-0726) were from Sigma (St. Louis, Mo). Antisera to C3 and human immunoglobulins IgG, IgM, and IgA were purchased from Cappel Laboratories, (Cochranville, Pa). Antiserum to laminin was kindly provided by Dr. George Martin, and antiserum to entactin was the generous gift of Dr. A. E. Chung.¹⁹ Monoclonal antibodies developed in this laboratory to fibronectin and Type IV collagen have been reported elsewhere.²⁰ Monoclonal antibodies to renal glycoproteins from human tumor cell lines^{21,22} were a kind gift from S. Singer (Ortho Diagnostic Systems, Raritan, NJ). All other chemicals were reagent grade.

Tissues

Human tissues were obtained from renal biopsies performed for clinical indications or nephrectomies done in preparation for renal transplantation. A total of 75 biopsies from 65 patients were examined for WGA binding. All tissues were snap-frozen in isopentane and stored at -70°C . Three- to four-micron frozen sections were cut with a cryostat and stained within 1 week by standard techniques.²³ Labeled lectins were used for staining in an appropriate dilution, usually 0.2–1.0 mg/ml. Because glycerol is known to interfere with some lectin binding, all tissues were mounted in 0.01 M phosphate-buffered saline, a coverslip was applied, and the sections were examined within 24 hours.¹⁵

Dual-label studies were performed by applying fluorescein-conjugated antibody first; and after adequate rinsing, the sections were stained with the rhodamine-conjugated lectin. Slides were viewed and photographed on a Zeiss epifluorescence microscope with appropriate fluorescein and rhodamine filters as described earlier.²³

Results

Frozen sections from normal human kidneys bound the lectins in a pattern qualitatively identical to that reported by Holthofer and co-workers.¹⁷ WGA reacted with the glomerular epithelium, mesangium, endothelium, and glomerular basement membrane as well as selected tubular epithelial cells at their apical brush

border and some distal tubular basement membranes (TBMs). Typically, normal human kidneys contain either no C3 or display only minimal fine granules of C3.

In MPGN-II the assessment of lectin binding is complicated by disease-altered morphology and an appreciation that even normal structures may be biochemically modified. Mesangial expansion leads to a quantitative lessening in reactivity with the soybean, *R communis*, and wheat germ lectins. *U europaeus* lectin binding to endothelium was markedly reduced by sclerosis and capillary collapse. Finally glomerular crescents moderately bound PNA. In spite of these differences, the overall qualitative patterns of lectin reactivity are not altered in MPGN-II.

Of all lectins examined, only WGA binds directly to the DIMD in MPGN-II. From 11 patients, with ultrastructurally proven MPGN-II, 21 separate biopsies of renal tissue were examined by dual-label fluorescence microscopy. Specimens were sequentially stained with fluorescein-conjugated antibody to C3 and then rhodamine-labeled WGA. Because WGA does not interact with complement nor immunoglobulin, the sequence of staining did not affect the staining pattern, a result also noted by others.¹⁷

All specimens from MPGN-II kidneys exhibited the characteristic changes of mesangial expansion and extensive C3 deposition. Normal WGA reactivity was variably recognized in these diseased kidneys but was altered proportionately to the extent of glomerular and tubular damage. Of the 21 specimens examined, 16 showed striking staining of DIMD by WGA, and at least one sample from each of the 11 patients with MPGN-II clearly demonstrated binding of WGA. Frequently the C3 outlined the dense deposits, producing "mesangial rings" and "railroad tracks." In addition, Bowman's capsule and TBMs contained numerous deposits outlined by C3. By dual staining, in each deposit outlined by C3, the central portion was uniformly reactive with WGA (Figure 1). Although slightly less intense, succinylated WGA also bound to DIMD; studies with all other fluorochrome-conjugated lectins (Table 1) revealed no binding to the dense deposits.

Additional specimens examined from normal human kidneys (3 patients), transplanted kidneys (16 patients), end-stage kidneys (13 patients), and kidneys affected by Type I MPGN (12 patients), diabetes (7 patients), amyloid (1 patient), and acute glomerulonephritis (3 patients) were entirely negative for membrane-oriented deposits binding WGA.

Prior incubation of WGA with 0.1 M NAG abolished all WGA reactivity with DIMD. However, pretreatment of the tissue sections with pronase, trypsin, collagenase, or neuraminidase (Figure 2) failed to reduce the staining. Similarly, tissue section incubation in either sul-

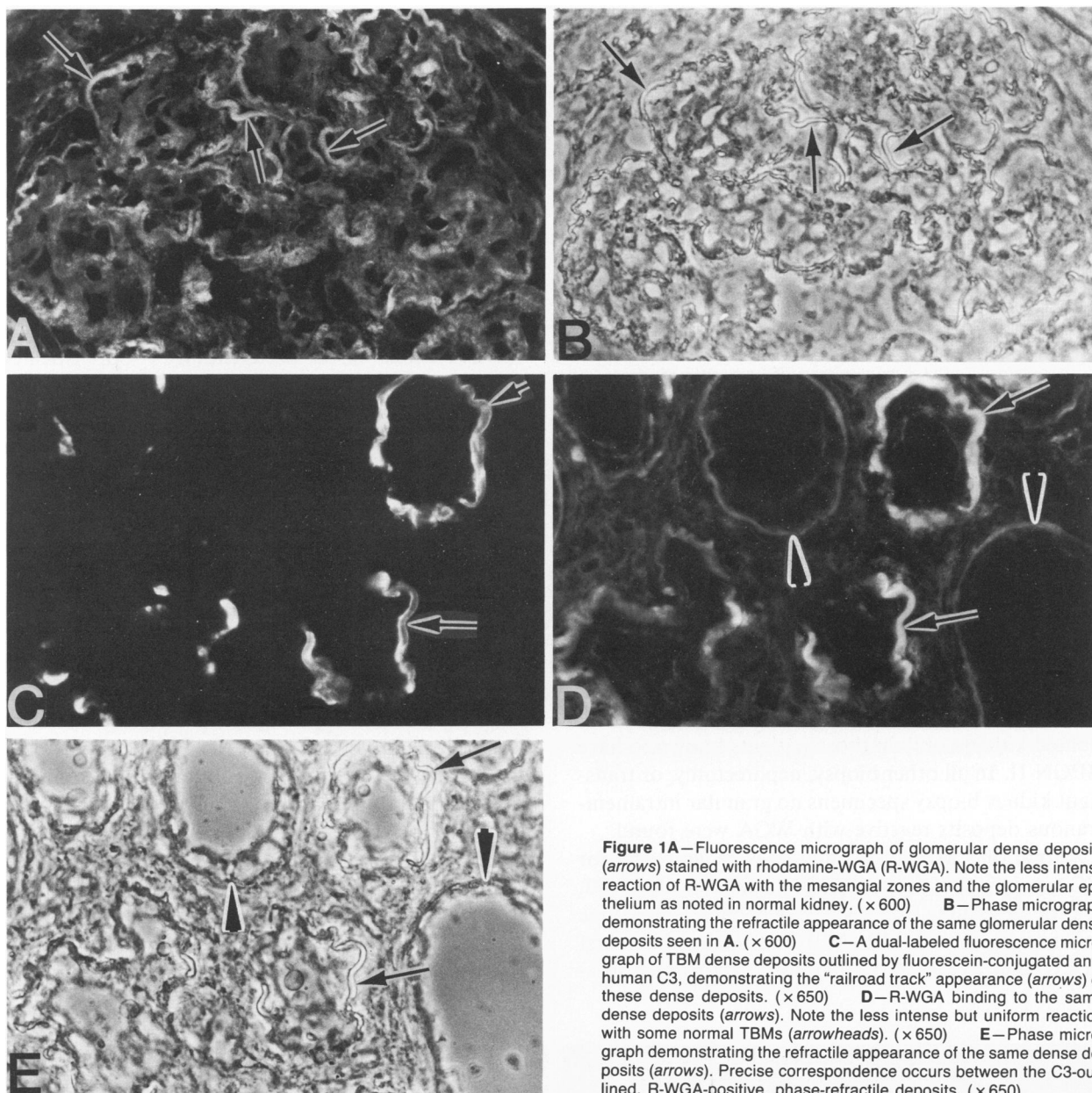


Figure 1A—Fluorescence micrograph of glomerular dense deposits (arrows) stained with rhodamine-WGA (R-WGA). Note the less intense reaction of R-WGA with the mesangial zones and the glomerular epithelium as noted in normal kidney. ($\times 600$) **B**—Phase micrograph demonstrating the refractile appearance of the same glomerular dense deposits seen in A. ($\times 600$) **C**—A dual-labeled fluorescence micrograph of TBM dense deposits outlined by fluorescein-conjugated anti-human C3, demonstrating the “railroad track” appearance (arrows) of these dense deposits. ($\times 650$) **D**—R-WGA binding to the same dense deposits (arrows). Note the less intense but uniform reaction with some normal TBMs (arrowheads). ($\times 650$) **E**—Phase micrograph demonstrating the refractile appearance of the same dense deposits (arrows). Precise correspondence occurs between the C3-outlined, R-WGA-positive, phase-refractile deposits. ($\times 650$)

furic acid (0.1 M) or desoxycholate (1%) did not reduce the WGA reactivity with dense deposits. However, it was noted that pretreatment with desoxycholate, sulfuric acid, or neuraminidase did reduce or abolish the normal pattern of WGA binding to glomerular cells.

Fluorochrome-conjugated WGA did not stain amyloid or the granular immune deposits in biopsies from Type I MPGN or acute glomerulonephritis. Rarely, there were examples of TBM staining where C3 and WGA were homogeneous and coincident; usually this was noted in kidneys with very thickened TBMs, as seen in diabetes.

Studies with antisera to immunoglobulin, comple-

ment, laminin, entactin, and monoclonal antibodies against fibronectin, type IV collagen and human renal tumor glycoproteins all failed to identify any of these antigens in the DIMD of MPGN-II.

Discussion

MPGN-II is a disease characterized by the accretion of DIMD in renal basement membranes. WGA binds discretely to these deposits occurring in glomerular basement membrane (GBM), TBM, or Bowman's capsule. DIMDs also bind thioflavin T,²⁴ are outlined by C3, and appear as refractile deposits by phase micros-

Table 1—Lectins Reactive With Dense Deposits in Type II MPGN

Lectin	Primary carbohydrate specificity	Reactive deposits*
WGA	β -NAG	+
Succinylated WGA	β -NAG	+
B simplicifolia II	α,β -NAG (terminal)	—
B simplicifolia I-B4	α -D-Galactose	—
R communis I	β -D-Galactose	—
PNA	β -D-Galactose	—
Con A	α -D-Mannose	—
UEA	L-Fucose	—
DBA	α -D-N-Acetylgalactosamine	—
Soybean agglutinin	α -D-N-Acetylgalactosamine	—

* Deposits identified by phase microscopy and outlined by antisera to C3.

copy. However, in each of these situations there may be some equivocal or contradictory findings.²⁵ Combining these techniques with the carbohydrate selectivity of WGA offers a confirmatory marker for DIMDs by phase-fluorescence microscopy.

In normal renal tissue as well as a variety of diseased kidneys, WGA binding by dense deposits was noted only in kidneys affected by MPGN-II. WGA binding was serially documented in biopsies of "native" kidneys, in "end-stage" specimens, and, finally, recurring in transplanted kidneys only in those patients known to have MPGN-II. In all other biopsy, nephrectomy, or transplant kidney biopsy specimens no granular intramembranous deposits reactive with WGA were found.

A total of 5 MPGN-II biopsies were equivocal for WGA staining but contained significant deposits of C3. Four of these came from a single hypocomplementemic patient with proven MPGN-II and included her first biopsy after 2 years of the nephrotic syndrome. Subse-

quently she required renal transplantation and underwent four additional biopsies. A later biopsy clearly showed WGA binding, but three others were ambiguous. The other indeterminate biopsy was obtained from a 16-year-old boy with MPGN-II following his third renal transplant. Previous and subsequent transplant kidney biopsies (4 biopsies in total) from this patient demonstrated distinct WGA-positive deposits. Experience with these 2 patients indicates that while WGA reactivity with DIMDs is consistent, there may be some technical variability in precisely identifying the smaller DIMDs at the phase-microscopic level in every biopsy.

WGA binds NAG preferentially.²⁶ Sophisticated binding studies further indicate that the binding is enhanced for NAG polymers containing up to three sequential residues.²⁷ Sterically, this extended binding site on WGA is capable of recognizing and interacting with NAG located either internally or terminally in the carbohydrate sequence. By contrast, *B simplicifolia* II lectin, which only interacts with terminal NAG residues,^{7,28} does not bind to the DIMDs.

Because of similarities of molecular conformation between sialic acid and NAG, WGA is also capable of binding certain sialoproteins.^{29,30} Usually sialic acid residues terminate a glycoprotein chain and are linked by galactose to internal sugars, principally mannose, N-acetylgalactosamine, and NAG. Frequently, acid hydrolysis of the sialic acid or treatment with neuraminidase results in a loss of WGA affinity, indicating the cooperation between internal and terminal sugars required to satisfy the trivalent WGA binding site. In MPGN-II biopsies, prior neuraminidase digestion did not reduce the intensity of binding between WGA and the DIMD. Additionally, since sialic acid usually is linked to a penultimate galactose residue, removal of

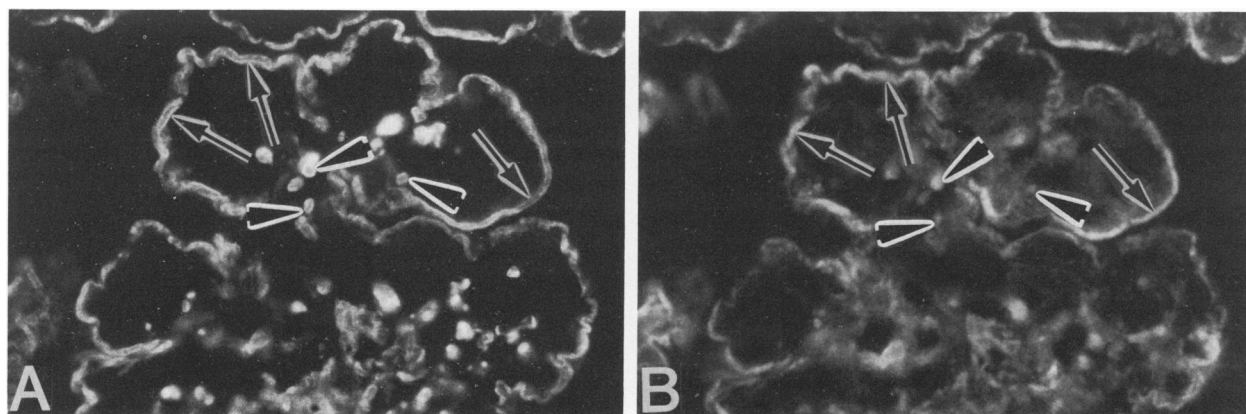


Figure 2—Dual-labeled fluorescence micrograph of a glomerulus following neuraminidase pretreatment. A—Fluorescein-conjugated anti-human C3, note the "railroad track" (arrows) appearance of membrane-oriented deposits and the appearance of "mesangial rings" (arrowheads). ($\times 800$) B—Rhodamine-conjugated WGA binds to the same deposits outlined by anti-human C3 in A. ($\times 800$)

sialic acid regularly leads to a polysaccharide molecule with a terminal galactose that strongly binds PNA. Again, either before or after neuraminidase digestion no interaction between the DIMD and PNA was demonstrated. Recently Monsigny and co-workers³¹ presented data supporting the concept that succinylated WGA specifically binds NAG but has minimal or no affinity for sialoglycoconjugates. On MPGN-II tissue succinylated WGA binds in a pattern identical to that of native WGA. In total, these studies indicate that internal NAG residues are a major carbohydrate component of DIMD and, further, suggest that sialic acid does not play a major role in the WGA-DIMD interaction.

Because WGA normally binds to renal glycoproteins, simple WGA binding to renal tissue is not pathognomonic for MPGN-II. Further, although WGA also reacts with certain normal serum proteins, (eg, α_1 -antitrypsin, α_1 -acid glycoprotein, etc.), it is reactive with neither C3 nor immunoglobulins. This characteristic permits WGA binding to discriminate the non-immunoglobulin-containing dense deposits in MPGN-II from the electron-dense immune deposits of glomerulonephritis.

In conclusion, the diagnosis of MPGN-II is elusive but critical, because these patients are subject to recurrent and potentially devastating disease in their transplanted kidneys.³² This study documents the selective binding of conjugated WGA to DIMDs and supports the use of WGA in identifying DIMDs in renal biopsies. This technique may be especially worthwhile in cases where inadequate specimens for electron microscopy or the absence of glomeruli on frozen sections further complicate the diagnostic process.

It is also possible that DIMD material may be isolated from affected kidneys by the use of immobilized lectin affinity columns and that this technique will permit further characterization of the DIMD.

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